GENESALB (2007-2010) : GENEtic analysis of resistance to South American Leaf Blight (SALB – *Microcyclus ulei*) in rubber tree (*Hevea* spp.)

Seguin M., Pujade-Renaud V., Rivallan R., Berger A., Déon M., Argout X., Goujon E., Doaré F., Le Guen V.
Garcia D.

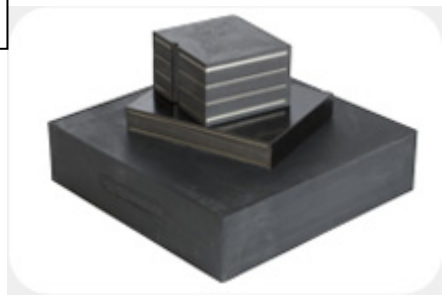
Granet F., Mattos C.R.R., Scomparin C., Fonseca F., Cubry P., Espéout S., Cavaloc E.

Seminar Plant Genomics 2012 – 4th April 2012 – Pont Royal en Provence

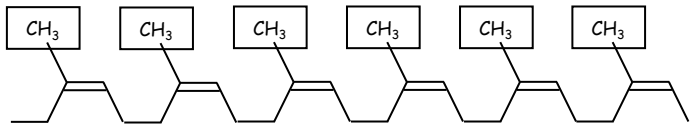
=> Abstract P78

Rubber tree (*Hevea brasiliensis*)

Rubber world consumption (24 Mt in 2010)
- 42% of natural rubber (*i.e.* from *Hevea*)
- 58% of synthetic rubber (petroleum)



Natural rubber: cis-polyisopren

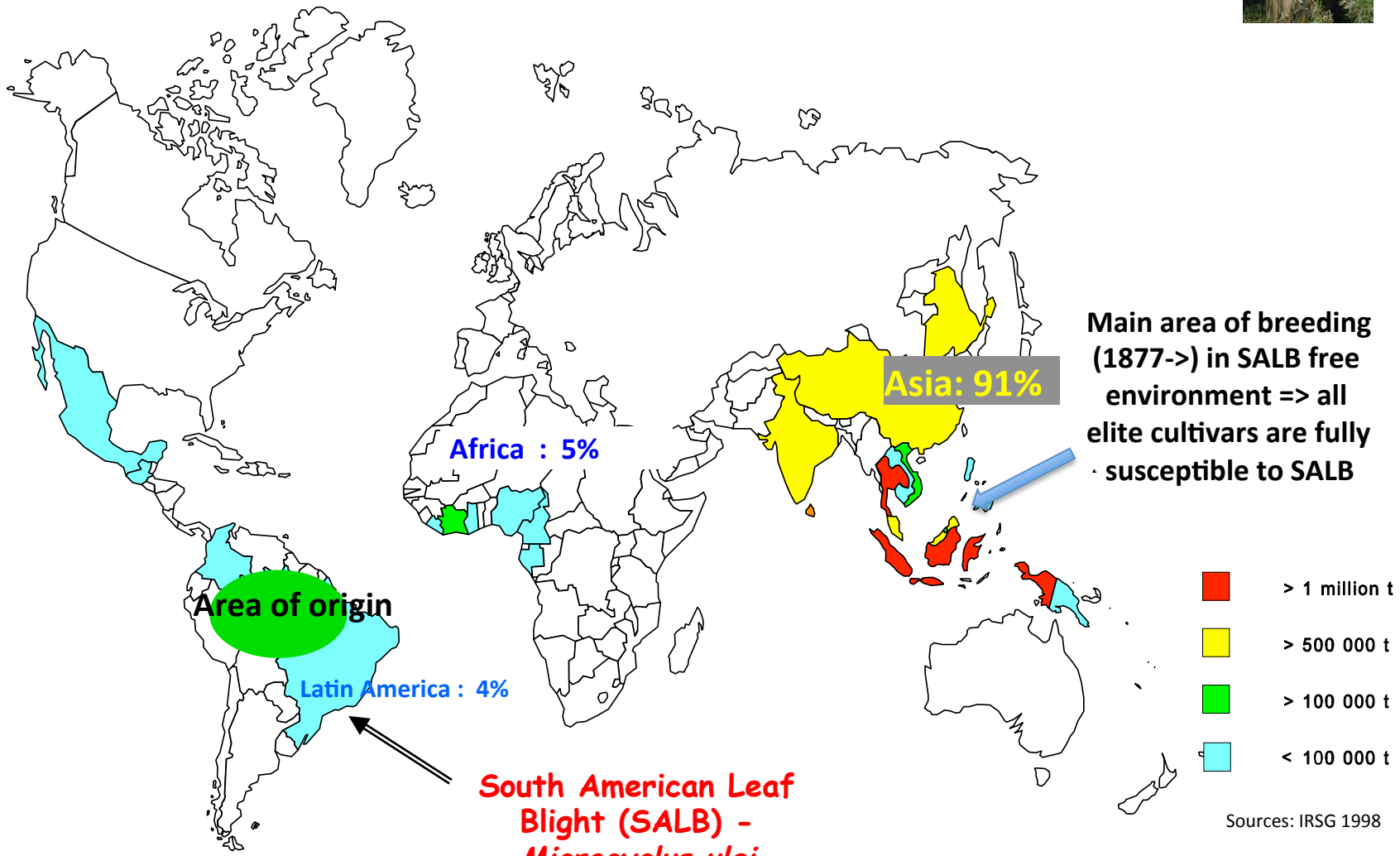


Rubber tree (*Hevea brasiliensis*)

- Rubber stored in latex = cytoplasm of laticiferous cells,
- Harvested by bark tapping, 2-3 times per week, during ~30 years

- 9 millions ha
- Production: 10,2 Mt (2010)
  - -> 80% by small holders
  - -> 20% by private companies

Rubber tree (*Hevea brasiliensis)*: areas of origin and of current production of natural rubber



Microcyclus ulei (Ascomycota):
repeated defoliations => death of the trees.

South American Leaf Blight:
a threat for world natural rubber production

1992 -> Cirad – Michelin partnership (CMB):

=> Create cultivars (clones) combining high yielding (latex) and resistance to SALB

genetic improvement of SALB resistance

- genetic mapping – genetic determinism
- phytopathology - epidemiology

2008-2010 GENESALB - ANR/Genoplante

- genetic mapping:

  diversity of genetic determinisms
- genomic resources
- for MAS development

G E N E S A L B : in connection with the Cirad – Michelin breeding program

SALB resistance:
- field evaluation
- controlled inoculations

- Hand pollination
- SALB resistance:
- field evaluation

Clermont-Ferrand: co-coordination

cirad

Montpellier, Cirad, UMR-AGAP : agap
- Genetics & Genomics
  - genotyping
  - QTL mapping
  - genomic resources: SSR coll. ESTs, BAC library

SALB resistance:
-field evaluation

Genomics: ESTs

GENESALB project (2008-2012)
3 sub-projects:

1. Genetic mapping and QTLs detection => diversity of genetic determinism and markers for early selection (MAS)

2. Markers development => for mapping; expressed genes integration in genetic maps, search for co-location with resistance loci/QTLs

3. Disease related ESTs / SSH libraries cloning, sequencing and expression analyses => candidate genes

GENESALB sub-project 3: candidate genes identification

Cloning of ESTs differentially expressed during SALB infestation:

- **11 SSH** libraries from leaves:
  resistant *vs* susceptible or healthy *vs* infected
  at several times after controlled inoculation

- **6700 ESTs** cloned and sequenced

- **490 candidate genes**: putative functions and expression profiling by **macro-arrays**

Fabien DOARE
(Cirad - Guyane)

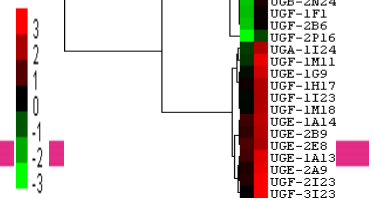
Valérie PUJADE-RENAUD

Angélique BERGER

Xavier Argout

(Cirad - UMR AGAP - Montpellier)

Dominique GARCIA
(Cirad – UESC – Bahia)



Garcia *et al.* (2011)

GENESALB sub-projects 2: markers development in ESTs and mapping

Search for SSR in ESTs sequences available:

- 760 SSR sequences identified
- 261 SSR screened for polymorphism by **bin mapping**
- **125 polymorphic EST-SSRs** identified
- 80 EST-SSRS mapped on GENESALB mapping populations


Philippe CUBRY (Michelin - Montpellier)


Valérie PUJADE-RENAUD


Marc Seguin


Vincent Le Guen

(Cirad - UMR AGAP - Montpellier)

Berger *et al.* in prep

GENESALB sub-projects 1: genetic mapping and QTL detection

Previous knowledge on genetic determinism of SALB resistance was based on only 1 mapping population:

- PB260xRO38: a multigenic resistance bypassed in French Guiana and Brazil

=> genetic mapping of 3 additional diversified sources (*Hevea* accessions) of resistance: MDF180, FX2784, FDR#

Carlos MATTOS & Fernando FONSECA (Michelin - Brazil)

Fabien DOARE (Cirad - Guyane)

Sandra ESPEOUT (Michelin - Montpellier)

Ronan RIVALLAN

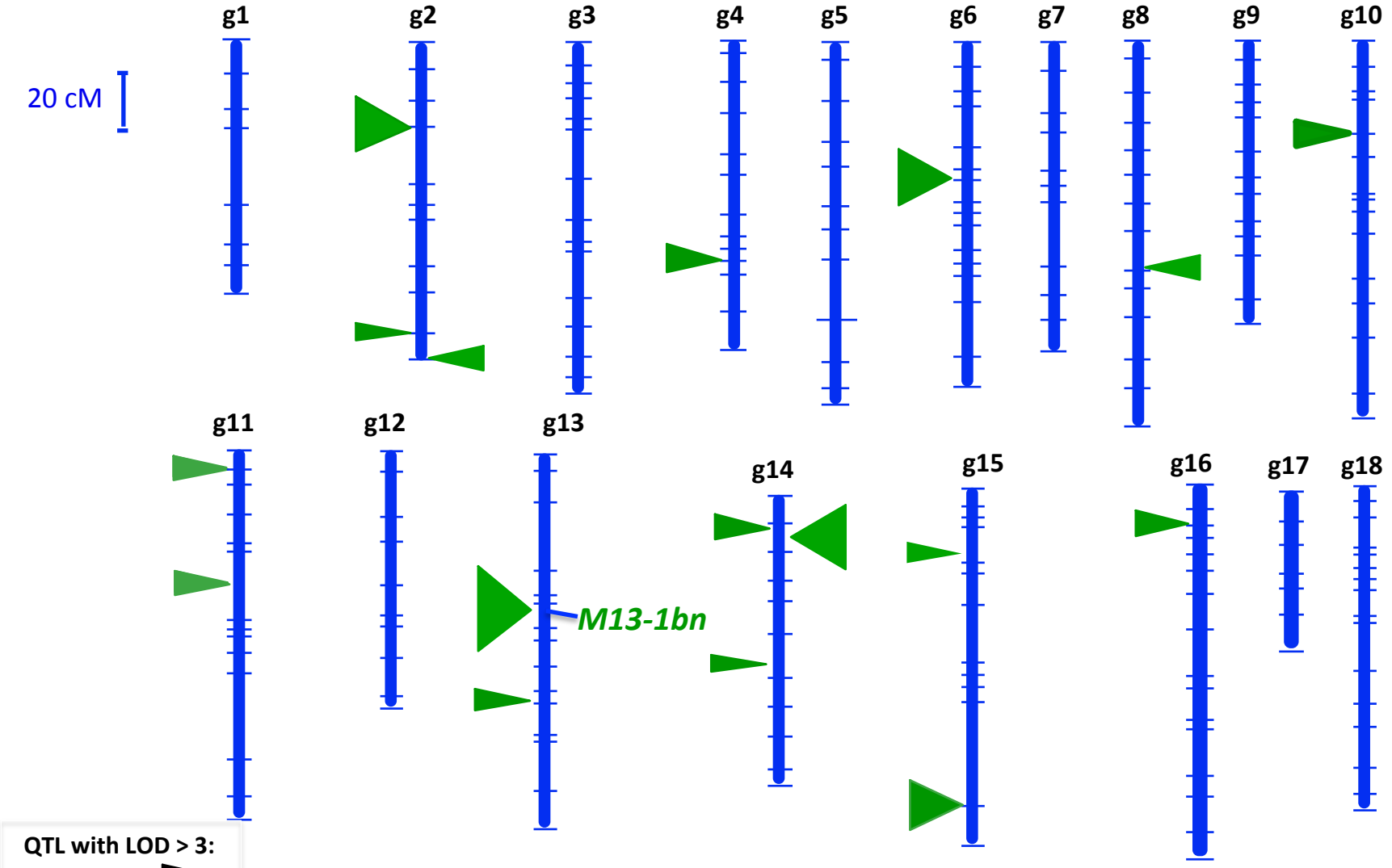
Dominique GARCIA

Marc Seguin

Vincent Le Guen

(Cirad - UMR AGAP - Montpellier)

Previous results :
Genetic determinism of SALB resistance of RO38:
a bypassed resistance despite its multi-genic determinism



***M13-1bn*: a major resistance locus governing qualitative resistance**

(Lespinasse et al. 2000, Le Guen et al 2007)

Qualitative trait loci (major genes) and Quantitative Trait Loci (QTLs) identification and mapping
- resistance evaluation in field trials -

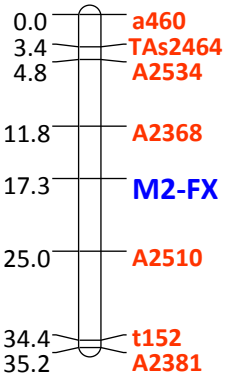
Mapping pop. Susceptible x Resistant	Field location	pop. size	markers	Resistance
PB260 x MDF180	French Guiana	351 F1	gSSR, AFLPs	durable in Brazil & French Guiana
PB260 x MDF180	Bahia	171 F1	gSSR, EST-SSR	
PB260 x FX2784	French Guiana	125 F1	gSSR	bypassed in Bahia
PB260 x FX2784	Mato Grosso	295 F1	gSSR	
PB235 x FDR#	Bahia	200 F1	gSSR, EST-SSR	durable in Brazil & French Guiana
PB260 x RO38	*French Guiana*	*500 F1*	*RFLP, AFLP gSSR*	*bypassed in Brazil & French Guiana*

PB260xFX2784 progeny in Mato Grosso (Plantation Edouard Michelin, PEM)

Carlos MATTOS & Fernando FONSECA (Michelin - Brazil)

<u>Preliminary result (Cirad)</u>: in French Guiana, FX2784 exhibit a **qualitative resistance** governed by a single resistance locus (2nd major locus, we named *M2-FX*) located on linkage group (LG) g2

PB260xFX2784
Linkage map
LG g2



Resistance locus integrated in the map as a genetic marker

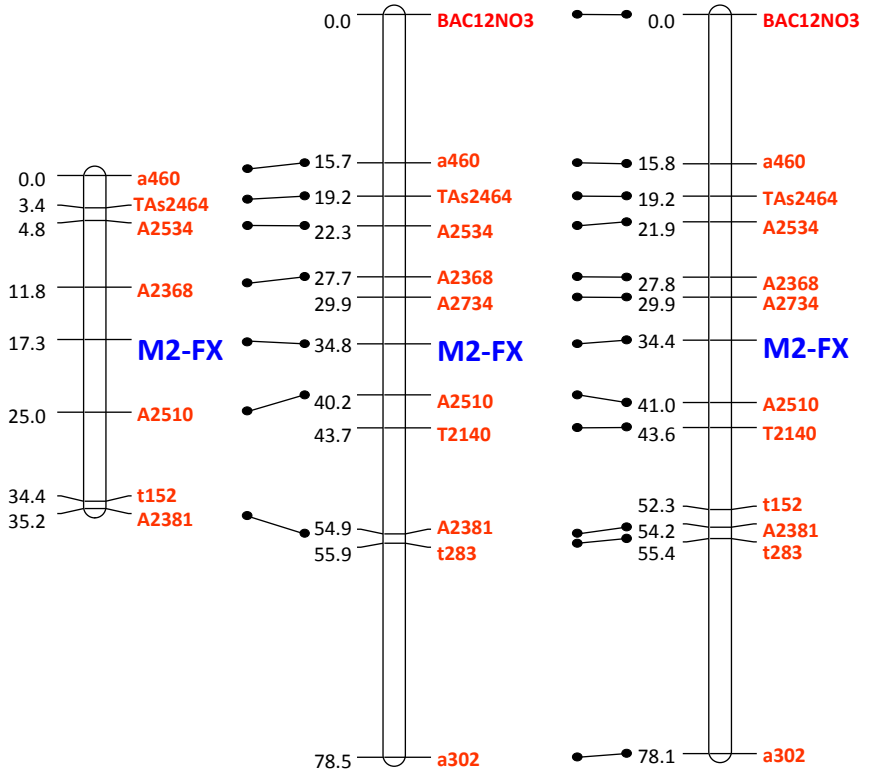
Microsatellite / SSR markers
Resistance locus / marker

GENESALB : identification and mapping of the **same *M2-FX* locus** governing qualitative resistance toSALB of **FX2784 in Brazil (Mato Grosso)**
- this resistance is bypassed in Bahia state -

PB260xFX2784
g2
French Guiana
125 F1

PB260xFX2784
g2
Mato Grosso
295 F1

Synthetic map
Guiana + Mato Grosso
420 F1



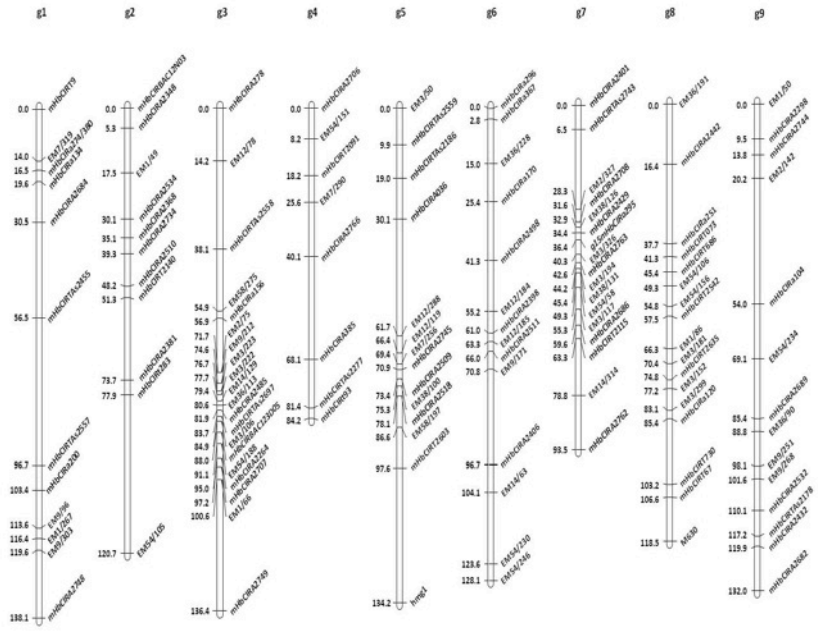
⇒ reliability and accuracy of field resistance evaluations

Microsatellite / SSR markers
Resistance locus / marker

Le Guen *et al.* In prep

GENESALB sub-project 1: construction of saturated maps for MDF180 and FDR# resistant clones (gSSR & EST-SSR markers)

MDF180 genetic map covering the 18 linkage groups / chromosomes



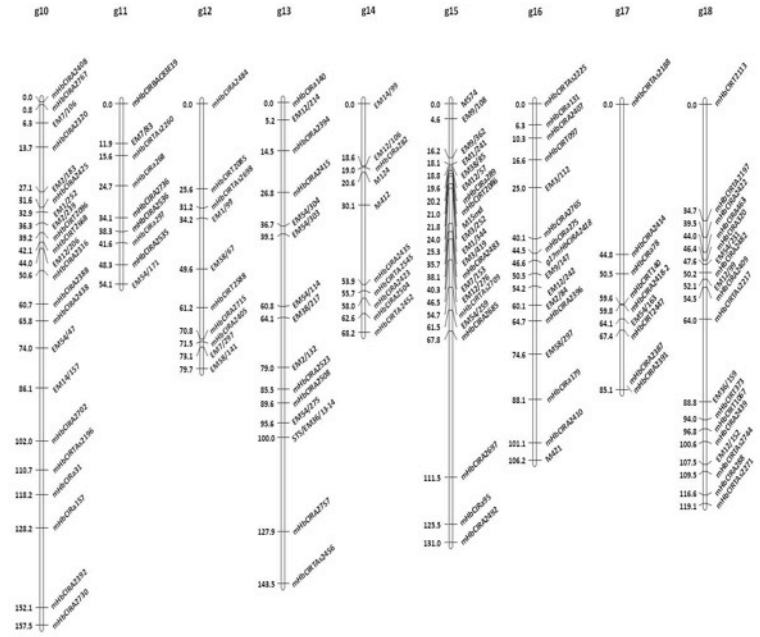
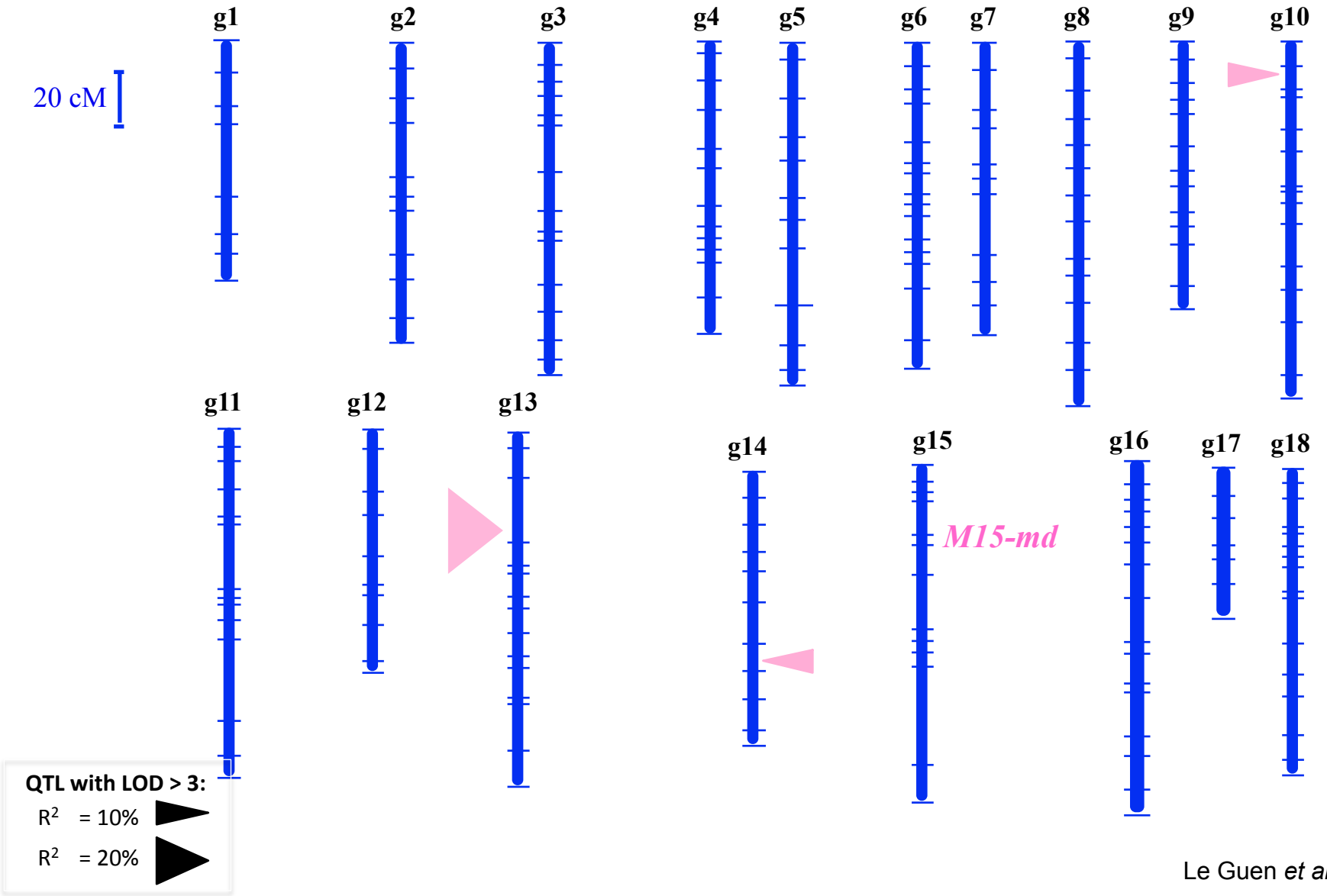


Fig. 3 MDF 180 linkage map of 155 microsatellite, 60 AFLP and 1 STS markers, distributed in 18 linkage groups

Fig. 3 (continued)

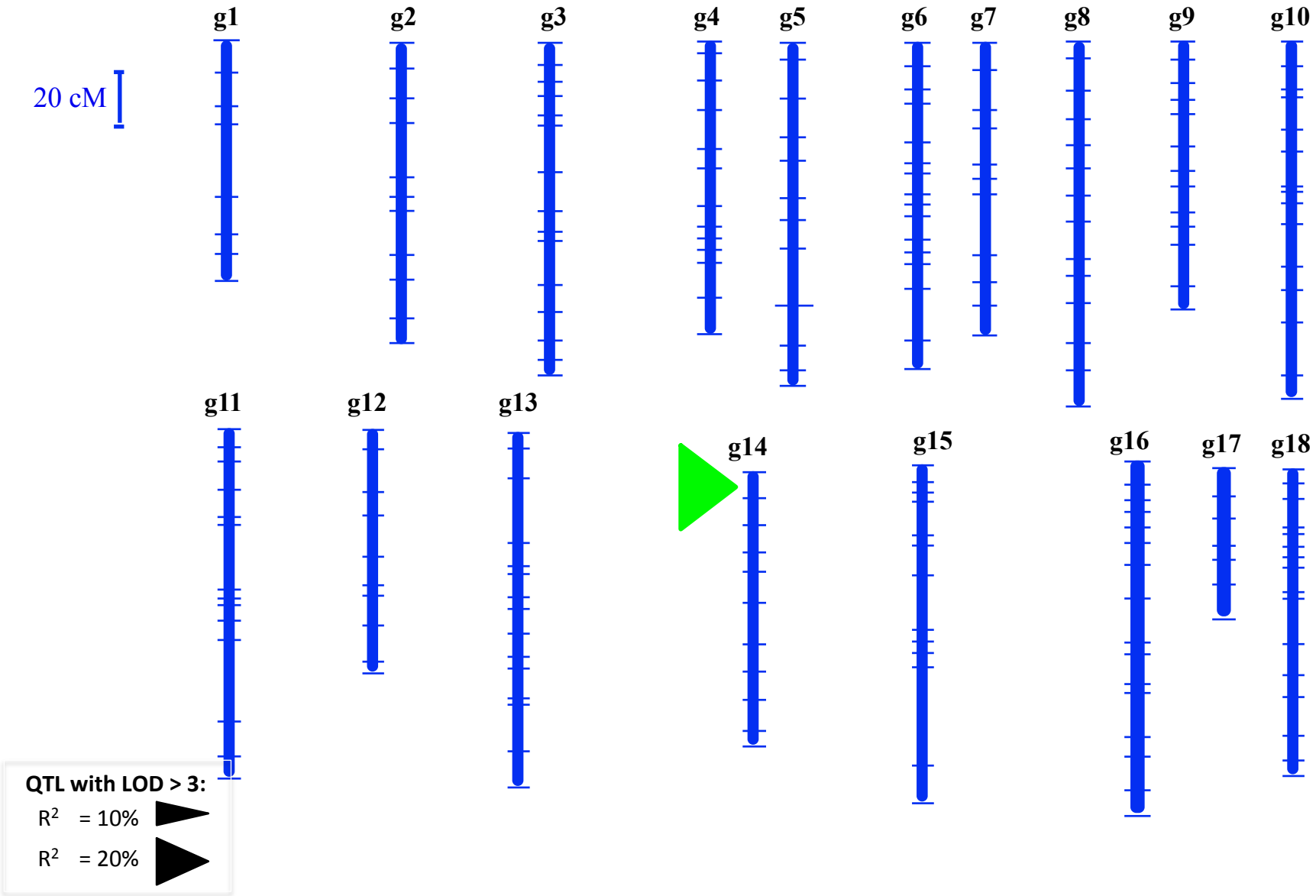
Le Guen *et al.* 2011

GENESALB : Genetic determinism of SALB resistance of **MDF180**:
a durable resistance governed by only 2 major loci
- identification of a 3rd major / qualitative resistance locus ***M15-md*** -

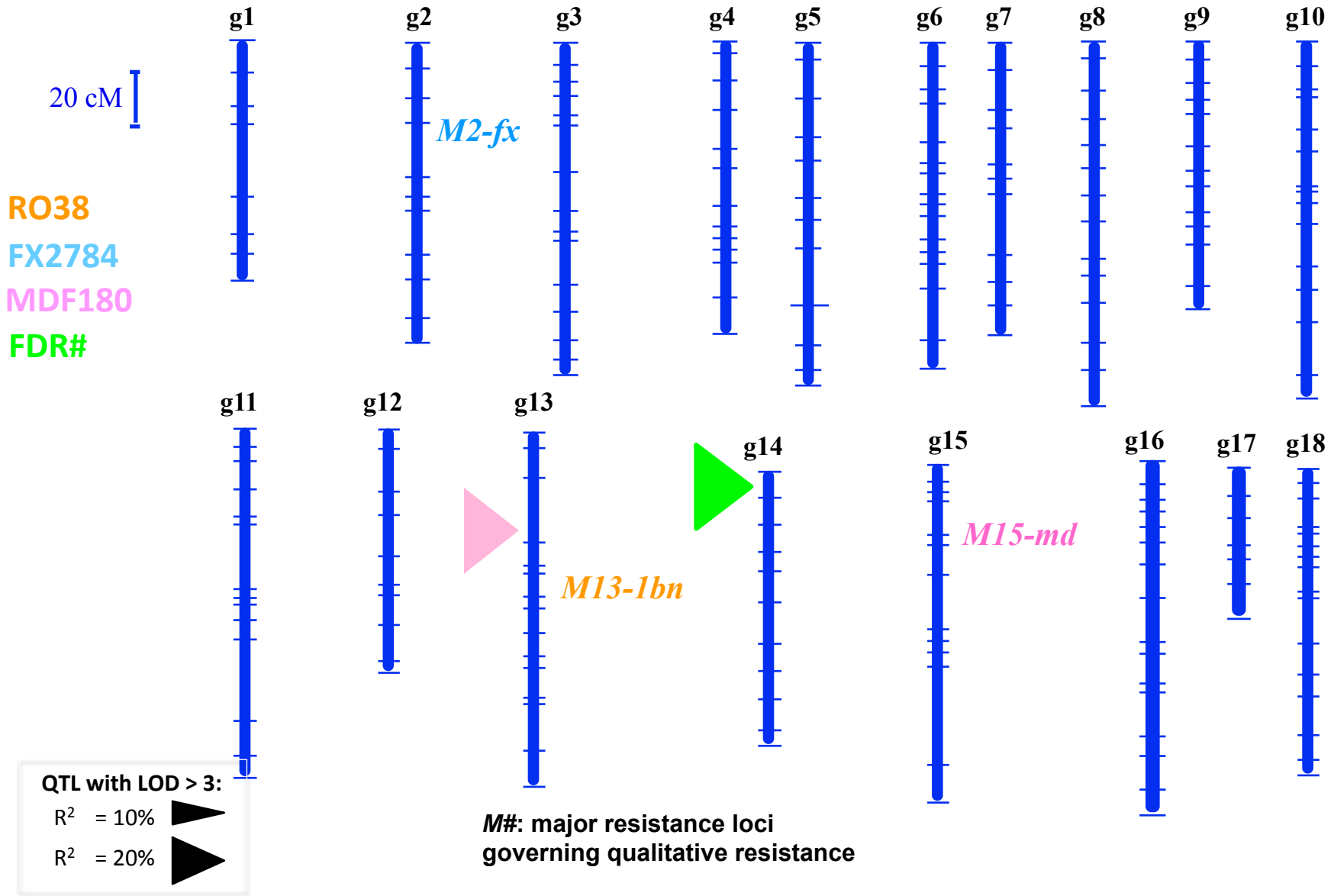


Le Guen *et al.* 2011

GENESALB : the durable resistance of **FDR#** is governed by a single major QTL in Brazil (Bahia)



GENESALB : simple genetic determinisms of resistance to SALB in rubber tree with a great diversity of resistance factors (qualitative R loci or QRLs)



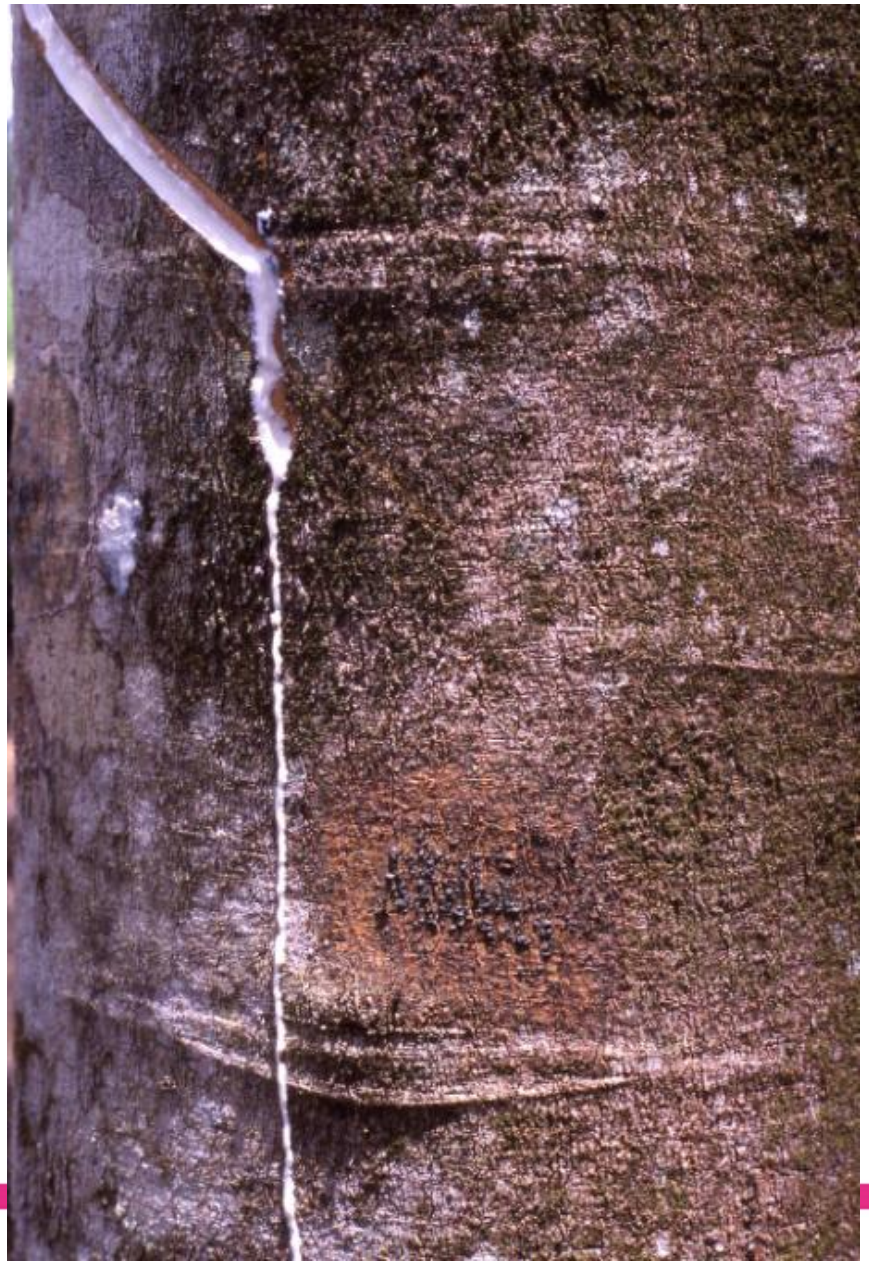
Conclusions / original results:

- Richness of genetic resistance factors to SALB in *Hevea* **genetic resources**
- **Simple genetic determinisms** of even durable resistances (do not fit with classical models !)
- **Four unlinked major resistance loci**
- A favorable situation for **resistance introgression and pyramiding** in few generations of inter-crosses
- Additional **genomic resources** for genetic, breeding and functional genomic studies

Prospects

- Development of marker aided selection (**MAS**)
- Genetic analysis of **additional sources of resistance**
- **QTL analysis of latex yield** and growth vigor
- **SNP markers development** for expressed and candidate genes mapping
- Q-PCR **validation of candidate genes**

Thank you for your attention



Merci de compléter les zones bleues DANS LA LIMITE DE 2 PAGES (police Tahoma – 9)

GENOPLANTE GIS bV **P0**	Thematic: «Other Species» **ANR n°: «ANR-07-GPLA-017»** **Acronym: «GENESALB»** Total Cost: **«1 710 000»** euros Total Grant: **«441 830»** euros Date of beginning: **«01.01.2008»** – End: **«31.12.2010»**	ANR **Edition 2007**

« Genetic analysis of resistance to South American Leaf Blight (SALB) in rubber tree (*Hevea* spp.)»

Seguin M.[1], Granet F.[2], Cavaloc E.[2], Scomparin C.[2], Mattos C.R.R.[3], Fonseca F.[3], Cubry P.[4], Espéout S.[4], Rivallan R.[1], Berger A.[1], Déon M.[1], Argout X.[1], Le Guen V.[1], Goujon E.[5], Pujade-Renaud V.[5], Doaré F.[6], Garcia D.[7]

Organism, City:

[1] Cirad, Montpellier; [2] Michelin, Clermont-Ferrand; [3] Michelin, Ituberà (Bahia, Brazil); [4] Michelin, Montpellier; [5] Cirad, Clermont-Ferrand; [6] Cirad – Guyane; [7] Cirad & UESC, Ilhéus (Bahia, Brazil)

Aims

Hevea brasiliensis cultivation is nearly the only source of production of natural rubber (latex), an irreplaceable strategic biopolymer for various industrial sectors. Worldwide production is threatened by the South American Leaf Blight (SALB) due to the fungus *Microcyclus ulei* (Ascomycota). CIRAD and Michelin collaborate, since 1992, on a program (CMB, Cirad-Michelin-Brésil) aiming at the creation of new varieties (grafted clones), combining high latex yield and tolerance to SALB. The ultimate objective is both to allow rubber farming development in the American inter-tropical zone endemically affected by the disease, and to prevent the risk of accidental introduction of the pathogen in the current Asian and African producing regions.

The aim of the GENESALB project was to reinforce genetic mapping and candidate genes identification in order to speed up the characterization of genetics factors governing natural resistance to SALB and, ultimately, to set up a marker-aided selection program. Our strategy was based on the analysis of 6 mapping populations issued from 4 diversified tolerant progenitors, evaluated for SALB resistance in 2 locations (Brazil and/or French Guiana) in order to assess genetic diversity of resistance factors (major genes/loci and QTLs).

☐

Results

Our results show that SALB resistance is mainly governed by major resistance genes/loci and each of the cultivar analyzed by genetic mapping led to the identification of a specific major resistance gene. These original results constitute a favorable context for a marker-aided selection of cultivars combining high yield and high level of SALB tolerance. In addition, it illustrates the richness of wild Amazonian genetic resources and their usefulness for disease resistance improvement in rubber tree.

The project produced molecular resources for expression studies and breeding: cloning and sequencing of 6958 expressed genes associated with the disease, characterization of 489 candidate genes and set up of 125 additional genetic markers. The project strengthens the leadership of Cirad and Michelin on this topic, and helped generating new partnership in Brazil.

Perspectives

Genetic mapping allowed identification of resistance factors, but also of SALB resistant individuals with a potentially improved latex yield. Indeed, all the mapping populations we analyzed correspond to crosses between a high yielding, susceptible progenitor by a low yielding but tolerant one. Available genotyping data allow marker aided selection of the individuals, with the resistance alleles at the resistance loci, that have to be, next years, more precisely evaluated in larger scale trials for other traits of interest such as latex yield and growth vigor. Other segregating populations issued from different tolerant progenitors are available in Brazil and QTL mapping of this material might reveal additional SALB resistance loci. One of these populations shows a complete inheritance of SALB tolerance, with most of the progenies showing a high level of resistance. We plan to apply genetic mapping of this population, which allows performing QTL detection of yield or growth without phytosanitary treatments of the trees against *Microcyclus*.

Development of genetic markers in EST-SSH will be continued. Around 350 ESTs from the SSH libraries, and containing a SSR motif, remain to be tested for EST-SSR polymorphism and mapping. Development of SNP markers will be necessary in order to map a greater number of the candidate genes we identified.

Merci de compléter les zones bleues DANS LA LIMITE DE 2 PAGES (police Tahoma – 9)

Publications / Congress

Garcia D., Carels N., de Sousa L.A, Sizenando Andrade S.J., Pujade-Renaud V., Mattos C.R.R., Cascardo J.C.M. (2011) EST profiling of resistant and susceptible *Hevea* clones infected by *Microcyclus ulei*. Physiological and Molecular Plant Pathology, 76:126-136.

Le Guen V., Garcia D., Doaré F., Mattos C., Condina V., Couturier C., Chambon A., Weber C., Espéout S., Seguin M (2011). A rubber tree's durable resistance to *Microcyclus ulei* is conferred by a qualitative gene and a major quantitative resistance factor. Tree Genetics & Genomes, 7:877-889

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Seguin M, Granet F, Argout X, Berger A, Bouchata K, Cavaloc E, Cubry P, Doaré F, Espeout S, Fonseca F, Garcia D, Goujon E, Le Guen V, Pujade-Renaud V, Scomparin C, Weber C (2010) GENESALB: Genetic analysis of resistance to South American Leaf Blight – SALB (*Microcyclus ulei*) in rubber tree (*Hevea* spp.). *In: Plant Genomics Seminar 2010*. Pont-Royal en Provence, France, 29-31 Mars 2010. Poster P059, Abstract pp.142-143.

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Total permanent scientist

Cirad :
Xavier Argout
Angélique Berger
Fabien Doaré
Vincent Le Guen
Valérie Pujade-Renaud
Ronan Rivallan
Marc Seguin
Christelle Weber

Cirad/UESC :
Dominique Garcia

Michelin :
Raphael Bortolazzo
Eric Cavaloc
Fernando Fonseca
Françoise Granet
Carlos Mattos
Cassio Scomparin

Temporary contracts

Two 18 months positions (Michelin):
Philippe Cubry,
Sandra Espéout

MsD students:
Karima Bouchata (M2)
Marine Déon (M2)
Eric Goujon (M1 & M2)